IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
In re application:
Masakazu HATANO et al.

U.S.Patent Application Number: 10/535,000 Group Art Unit:1612
Filed: Nov 17, 2003 Examiner: Huang, Gigi, Georgiana
For: THERAPEUTIC AGENT FOR GLAUCOMA COMPRISING Rho KINASE INHIBITOR AND β-BLOCKER

DECLARATION UNDER 37 CFR 1.132

Honorable Commissioner
of Patents and Trademarks
Washington, D.C. 20231
Sir:

I, Masakazu Hatano, a citizen of Japan, hereby declare and state:

1. I am a joint inventor in the above identified application, and am a Japanese citizen, residing higashiyodogawa-ku, Osaka-shi, Osaka 533-0032 Japan.

2. I have a master's degree which was conferred upon me by Kyushu University, Faculty of Pharmacy in 2000.

3. I have been employed by Santen CO., LTD., Japan, the assignee of record in the present application. Since 2000, I have been engaged in that corporation and have had a total of 9 years of work and research experience in pharmacology.

4. I am very familiar with the present invention, the above-identified application, the Office Actions dated on November 26, 2008 and May 28, 2009 and reference cited therein,

5. I and/or those under my direct supervision and control carried out pharmacological test to study intraocular pressure lowering action of a Rho kinase inhibitor, 1-(5-isoquinolinesulfonyl)-homopiperazine, and a β blocker, timolol, in combination. I would like to report the results of the pharmacological tests below.

Description of Procedure for Pharmacological Tests

1. Preparation of Test Compound Solutions

A Rho kinase inhibitor, 1-(5-isoquinolinesulfonyl)-homopiperazine (also known as "HA1077"), was dissolved in physiological saline to prepare 0.3% HA1077 solution (hereinafter referred to as "HA1077 solution"). For β-blocker, a commercially available ophthalmic solution containing timolol as an active ingredient (trade name: Timoptol) was used as 0.25% timolol solution (hereinafter referred to as "timolol solution").

2. Method of Test

After the combination of HA1077 and timolol was administered to experimatal animals, the effect on reducing intraocular pressure (IOP) was studied. As a reference, after HA1077 alone or timolol alone was administered, the effect on reducing IOP was also studied. As a control, only a vehicle (physiological saline) was administered. Japanese white rabbits (four rabbits per group) were used as experimental animals.

3. Method of Administration and Method of Measurement

1) Administration of the combination of HA1077 and timolol

IOP was measured immediately before administering the test compound solution, and the measured IOP was referred to as initial IOP. HA1077 solution was instilled into one eye of each experimental animal (the other eye was not treated). After a short period (about five minutes), timolol solution was instilled into the same eye. One, two and four hours after instilling HA1077 solution, IOP was measured three times to obtain the average of the three measurements.

2) Single administration of HA1077

Each test was carried out in the same manner as in the above-mentioned combination administration test except that timolol solution was replaced with physiological saline.

3) Single administration of timolol

Each test was carried out in the same manner as in the above-mentioned combination administration test except that HA1077 solution was replaced with physiological saline.

4) Control

Each test was carried out in the same manner as in the above-mentioned combination administration test except that HA1077 solution and timolol solution were replaced with physiological saline.

Results of Pharmacological Tests

The results of pharmacology tests are shown in Fig. A and Table A below.

Fig. A Effect of topical administration of HA1077 and timolol in combination on IOP in ocular normotensive rabbits

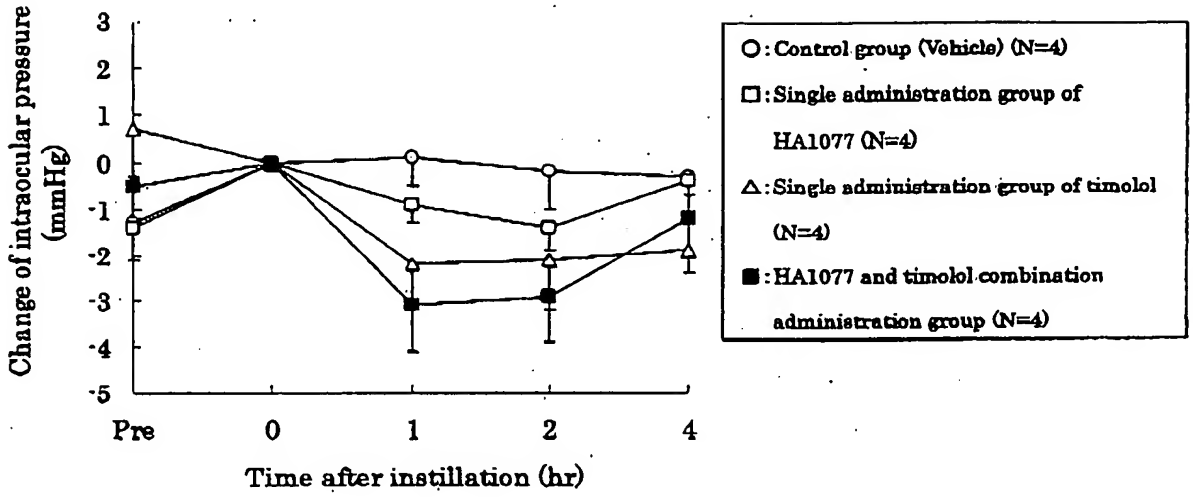


Table A IOP reduction from initial IOP after administration of HA1077 and timolol in combination as the difference from the control group

	Time after instillation			
	0 hr	1 hr	2 hr	4hr
①Single administration group of HA1077	0	1.0 mmHg	1.2 mmHg	0.1 mmHg
②Single administration group of timolol	0	2.3 mmHg	1.9 mmHg	1.6 mmHg
③HA1077 and timolol combination administration group	0	3.2 mmHg	2.7 mmHg	0.9 mmHg
Theoretical additive IOP reduction (①+②)	0	3.3 mmHg	3.1 mmHg	1.7 mmHg

Analysis of Results

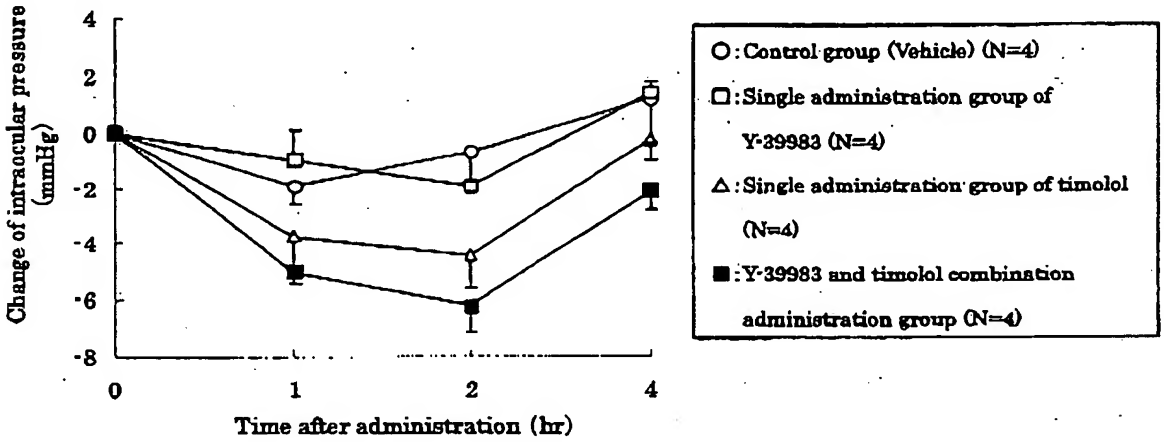
As apparent from Fig. A, HA1077 and timolol combination group exhibited additive effects 1 and 2 hours after instillation. However, 4 hours after instillation, HA1077 and timolol did not complement and/or enhance their actions with respect to each other, which means that persistence of the action was not improved by this combination.

Further, this combination did not exhibit synergistic effects at any point in time. Table A shows IOP reduction from initial IOP after administration of HA1077 and timolol in combination as the difference from the control group. According to Table A, IOP reductions of combination group 1, 2 and 4 hours after instillation are 3.2 mmHg, 2.7 mmHg and 0.9 mmHg, respectively. It should be noted that IOP reduction 4 hours after instillation are smaller than the theoretical additive IOP reductions while IOP reductions 1 and 2 hours after instillation are approximately equal to the theoretical one (See the bottom of Table A). Consequently, Table A supports the findings that the combination of HA1077 and timolol exhibits a partially additive effect, not a synergistic

one.

On the other hands, as described in "pharmcological tests" in the present specification, the combination of (R)-(+)-N-(1H-pyrrolo[2,3-b]pyridin-4-yl)-4-(1-aminoethyl)benzamide (also known as "Y-39983") as a Rho kinase inhibitor and timolol, improved persistence of the action. Fig. 1 of the present application is reproduced as follows.

Fig. 1 Effect of topical administration of Y-39983 and timolol in combination on IOP in ocular normotensive rabbits



Further, it should be noted that this combination exhibited a synergistic effect. To demonstrate these findings, calculating IOP reduction from initial IOP after administration of Y-39983 and timolol in combination as the difference from the control group, the results are shown in Table 1 below.

Table 1 IOP reduction from initial IOP after administration of Y-39983 and timolol in combination as the difference from the control group

	Time after instillation			
	0 hr	1 hr	2 hr	4hr
①Single administration group of Y-39983	0	-0.9 mmHg	1.2 mmHg	-0.2 mmHg
②Single administration group of timolol	0	1.8 mmHg	3.7 mmHg	1.4 mmHg
③Y-39983 and timolol combination administration group	0	3.1 mmHg	5.5 mmHg	3.3 mmHg
Theoretical additive IOP reduction (①+②)	0	1.1 mmHg	4.9 mmHg	1.2 mmHg

According to Table 1, IOP reductions of the combination group after 1, 2 and 4 hours after instillation are 3.1 mmHg, 5.5 mmHg and 3.3 mmHg, respectively. It should be noted is that IOP reductions at any point in time are greater than theoretical additive IOP reductions (See the bottom of Table 1). Consequently, Table 1 supports the findings

that the combination of Y-39983 and timolol exhibits a synergistic effect.

In conclusion, the combination of Y-39983 and β-blocker (i.e. timolol) improved persistence of IOP reduction while the combination of the other Rho kinase inhibitor such as HA1077 and β-blocker did not. Further, the combination of Y-39983 and β-blocker also exhibited a synergistic effect while the combination of the other Rho kinase inhibitor and β-blocker merely exhibited a partially additive effect.

The undersigned declares that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Signed this 21st day of July, 2009

Masakazu Hatano

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